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Attorney of Record

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant: Diana M. Downs, et. al.  
Appl. No.: 09/955,502  
Filed: September 18, 2001  
Title: METHOD FOR PREVENTING SUPEROXIDE DAMAGE TO CELLS AND OXYGEN-LABILE PROTEINS  
Art Unit: 1645  
Examiner: Patricia A. Duffy  
Atty. Docket No.: 960296.97559  
Client Docket No.: P01063US

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**DECLARATION OF INVENTOR DIANA DOWNS, PHD**

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1. I, Diana Downs, am an inventor in the above identified patent application. I am a professor in the Bacteriology Department, University of Wisconsin, Madison where I specialize in biochemical genetics and bacterial metabolism.

2. I have reviewed the currently pending office action and submit the following information in response to the Examiner's supposition that Applicants have only enabled a method of reducing superoxide damage in cells with endogenous expression of the YggX gene.

3. I, and those under my supervision, have performed the following experiment: We engineered the expression of either the YggX protein from E. coli or the YggX protein from Salmonella in Salmonella. Overnight cultures grown in rich media were subcultured with 150 µl added to 5 ml NB containing none or 20 µM paraquat as

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indicated. (Paraquat is a superoxide generator). Cultures were incubated with shaking at 37° C, and growth was monitored by OD650 using a Bausch and Lomb Spectronic 20. The final OD650 reported was after 24 hrs, since absorbance for all strains had reached a plateau by the time. Strains used were all Salmonella strains with YggX inactivated by insertion. Each strain contained one of three plasmids: (1) no insert control, (2) plasmid expressing the Salmonella YggX, (3) plasmid expressing the E. coli YggX.


4. I attach the data as Fig. 1. You will notice that the Salmonella protein works better, but at the 20 micromolar concentration the E. coli protein is clearly having the same effect.
5. The homologs described in above-identified application are the functional and structural equivalents of the YggX protein that we initially used in our experiments in large part because of their identity at the sequence (93%) and structural level. I note publication by several authors (Pomposiello et. al., J. Bact. 185:6624; Osborne, MJ et. al. Protein Sci 14:1673) that confirms this fact. In biology today, structural identity is equated extensively (and often without question by reviewers of primary manuscripts) with functional similarity. One of the primary reasons this is so is because of the consistency of basic metabolic paradigms across life forms.
6. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of

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the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Respectfully submitted,

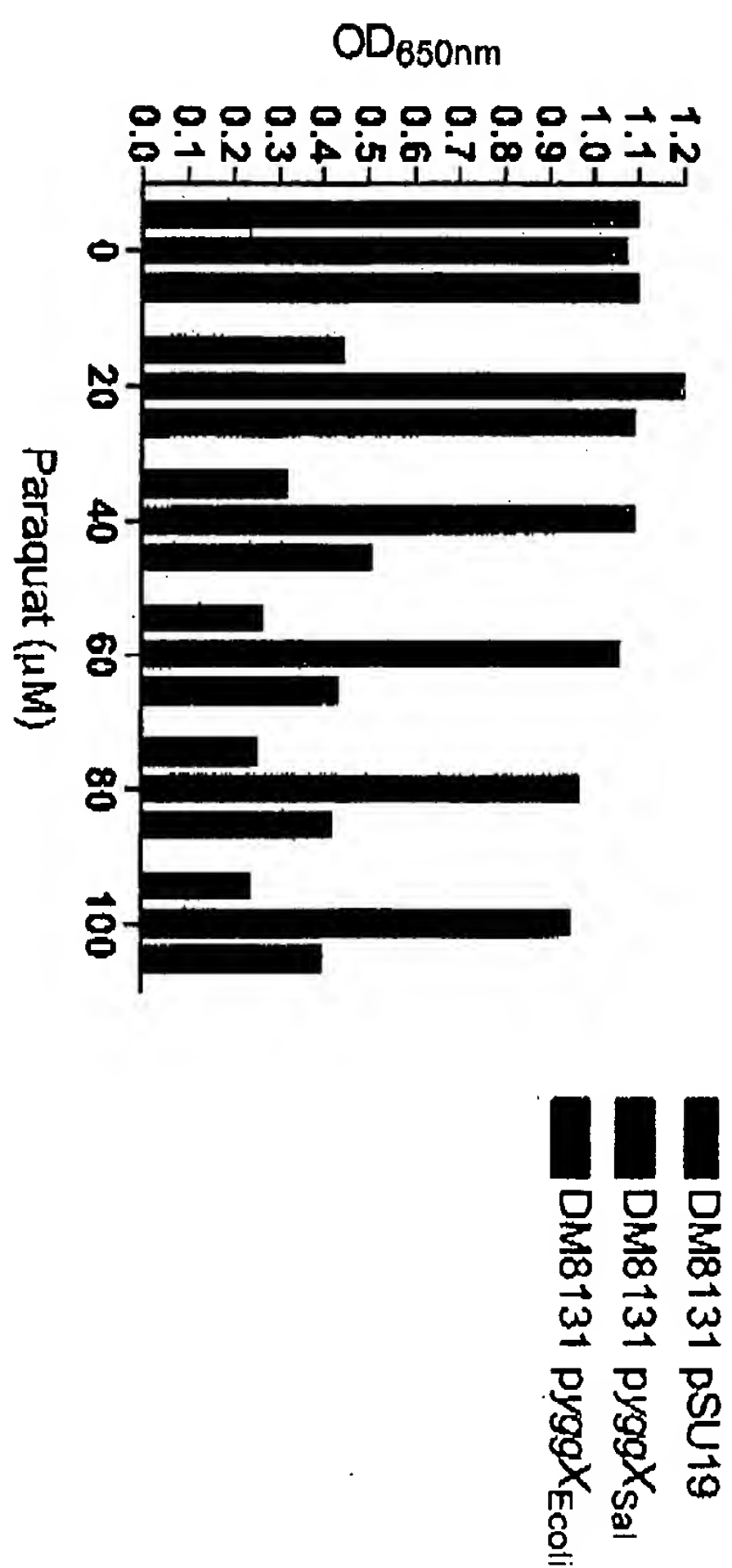
Dated: 12/22/05

  
Diana Downs, Ph.D.

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FIG. 1



Strain DM8131 is DM8000 with *yggX::kan* knock-out